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Models of Excitation-Secretion Coupling in Pituitary Cells

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This report describes initial progress in developing a biophysical description of the electrical events surrounding hormone release in pituitary cells. Utilizing a model based on a membrane capacitance shunted by potassium and calcium channels, we are developing a computer program that will simulate the dynamic response of the transmembrane potential of GH3 cells in response to thyrotropia-releasing hormone (TRH). When available, we are using published channel models of potassium and calcium channels. In parallel, we are persuing a phase-plane description of the electrical properties. These models and analyses will be used to investigate the effect of temperature on membrane action potentials.

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Principal Investigator: C. Frank Starmer, Ph.D.
Duke University Medical Center
10/25/89

Introduction:

Hormone release from pituitary cells appears related to a modification of the basal electrical activity of the cell. This electrical activity has been shown to be modulated by agents that also regulate secreting activity. For instance Thyrotropin-releasing hormone (TRH) triggers the release of prolactin in GH_3 cells and simultaneously leads to an increase in action potential frequency in these electrically active cells. The membrane potential associated with the appearance of released hormone in the extracellular fluid appears to initially be hyperpolarized (thought to result in opening of a Ca^+ activated potassium channel) followed by a decrease in the voltage dependent K^+ currents. Dubinsky and Oxford (1) have suggested that upon application of TRH 1: Ca^{++} is released from intracellular stores which activates Ca^{++} activated K channels; 2: voltage-dependent K channel openings are depressed during hyperexcitable phase and that 3: TRH does not directly modulate calcium channel activity. During the burst of action potentials during the hyperexcitable phase, extracellular Ca^{++} enters the cells through voltage gated Ca^{++} channels (perhaps to participate in prolactin secretion) (2) and accumulates to a point where electrical activity becomes again silent.

Temperature effects the transition rates of voltage gated channels (3,4). This project addresses the effect of temperature on models of the electrical properties of cellular action potentials and on the accumulation of intracellular Ca^{++}. The work has followed three directions: that of developing computing tools to facilitate management and display of model results; developing a minimal model of the electrical properties of GH_3 cells and to develop analytic tools to characterize the use-and frequency-dependent properties of the accumulation intracellular calcium during bursts of action potentials.

Progress Report:

1) Our research group has been involved in developing software tools to facilitate acquisiton, management, analysis and display of research data. The most recent work has focused on developing tools for visualizing primary data, results of simulations and displays of derived results. Using the X-window system operating on Sun 4 workstations, we have developed a graphical editor that allows cutting and pasting segments of a graphic display. These selected segments can be printed or subjected to further analyses e.g. curve fitting. In addition we have developed a tool for scanning a sequence of research data records (e.g. simulations for a set of different conditions). These tools have been extremely useful in visually investigating a small segment of a long simulation. The software tools have been described in several manuscripts listed at the end of this report (7,8).

2) GH_3 cell model. For a minimal model, we have considered a 3 component model: a voltage activated potassium current, I_k, and voltage and calcium inactivated calcium current, I_{ca}, and a leakage current, I_l, that are considered electrically in parallel with the membrane capacitance. In addition, we have assumed a first order sequestration of internal calcium. Defining the membrane capacitance as C_m we define the minimal model as

$$\frac{dv}{dt} = - \frac{1}{C_m} (I_{ca} + I_k + I_l)$$

where V is the membrane potential, I_{ca} is the calcium current described by

$$I_{ca} = \bar{g}_{ca}\, d(V)\, f\, (V, Ca_i)\, (V - V_{ca})$$

where d and f are activation and inactivation gating variables, V_{ca} is the calcium reversal potential and $\bar{g}_{ca}$ is the maximum calcium conductance. Similarly, for the potassium current

$$I_k = \bar{g}_k\, n(V)(V - V_k)$$

where n(v) is the activation variable, V_k is the reversal potential and $\bar{g}k$ is the maximum potassium current. The gating

variables are defined to reflect transitions in channel protein conformations according to a simple first order model

$$\text{closed} \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} \text{open}$$

so that for the potassium channel, n at equilibrium is $n = \alpha/\alpha+\beta$ and $n(t)$ is the solution to

$$\frac{dn}{dt} = \alpha\ (1 - n)\ -\beta\ n$$

To incorporate Ca^{++} inactivation into the inactivation variable f, we assume a first order process leading to an equilibrium inactivation of the form $f_\infty = [1 + Ca^{++}/k_n]^{-1}$ Finally, intracellular calcium distribution is determined by

$$\frac{d}{dt}Ca^{++} = I_{ca} - K_{ca}\ Ca_i^{++}$$

where I_{ca} represents calcium entering the cell through open channels and is normalized per unit volume. We have solved these equations with some preliminary estimates of rate constants and have investigate temperature dependence assuming a Q_{10} of 3. We have found that this simple model can exhibit 3 types of temperature sensitive behavior (figure 1 and 2): simple oscillations, bursts of oscillations and continuous oscillations from a depolarized baseline. These results suggest the nonlinear terms in the model can produce behavior similar to that seen in other systems exhibiting behavior described by the term, chaos. Figure 1 illustrates results when the absorption rate of Ca_i is held constant while the temperature effect is restricted to channel conformation rates. Figure 2 illustrates the same sets of rate constants but also allowing k_{ca} to vary with temperature. The vertical axis is membrane potential (mV) while the horizontal axis is time (msec). The detailed mechanism leading to such dramatic changes in oscillatory behavior is the focus of current investigations.

3) Our work with analytically characterizing the use-dependent properties of Ca_i has followed that of our models of ion channel blockade (5). Basically, with each action potential, intracellular calcium is incremented by a fraction, proportional to the difference between

intracellular and extracellular calcium. For the n^{th} action potential when the channel is conducting

$$\frac{d}{dt}C_i = \gamma(C_o - C_i) - k_a C_i$$

where C_i and C_o are intracellular and extracellular concentrations and γ represents diffusion rate down the calcium concentration gradient. When the channel is not conducting, C_i is reduced through intracellular storage at a rate k_a so

$$\frac{d}{dt}C_i = -k_a C_i$$

If the channel open time is exponentially distributed with mean, t_o, (5) then

$$C_i = C_{(\infty)} + [C(o) - C(\infty)]\ e^{-(\gamma C_o + k_a)t_o}$$

where $C(\infty) = \gamma C_o/(\gamma + k_a)$

While the channel is not conducting

$$C_i\ (t) = C(o)\ e^{-k_a t}$$

During a burst, internal calcium is incrementally increased so that for the n^{th} pulse

$$C_n = C_{ss} + (C_o - C_{ss})e^{-[k_a t_r + (\gamma + k_a)t_o]n}$$

where t_r is the interpulse interval between action potentials during a burst and t_o is the mean channel open time and

$$C_{ss} = \frac{C_{(\infty)}(1 - e^{-(\gamma + k_a)t_o}}{1 - e^{-[k_r t_r + (\gamma + k_a)t_o]}}$$

Thus, it is possible to estimate the behavior of intracellular calcium during and between bursts if the channel open time is exponentially distributed. These preliminary analyses will be extended in an attempt to capture some of the chaotic features of channel bursting, and to compare analytical results with those derived from numerical simulation.

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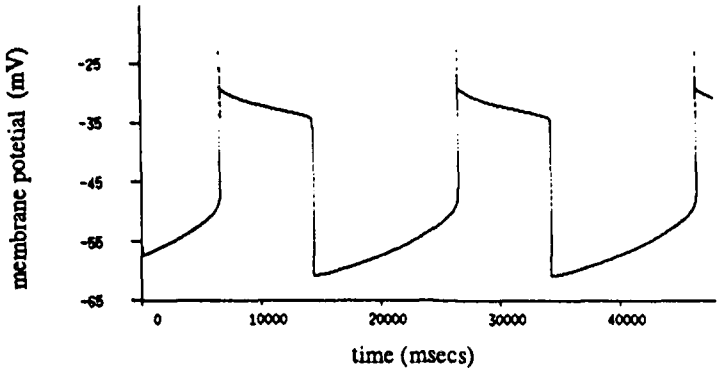

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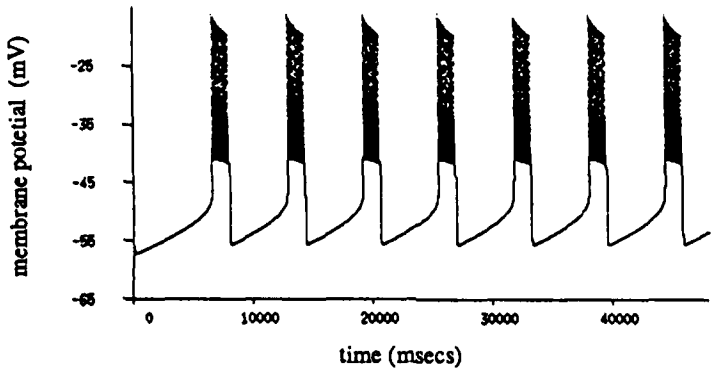

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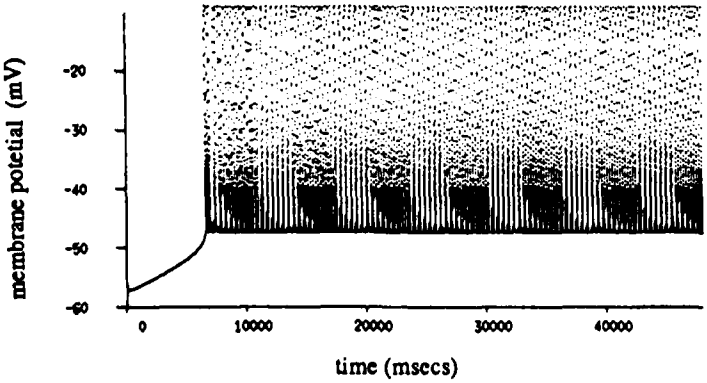

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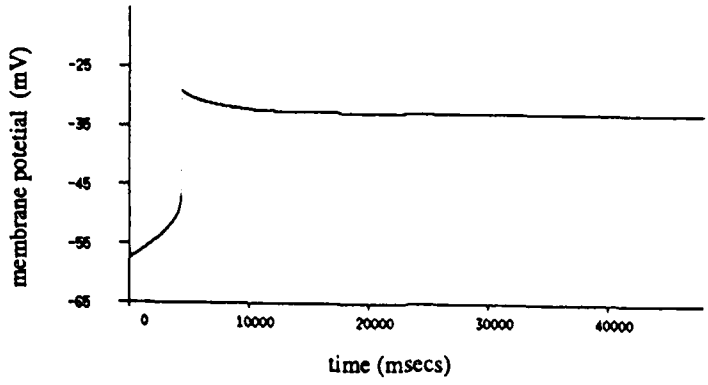
Figure 1



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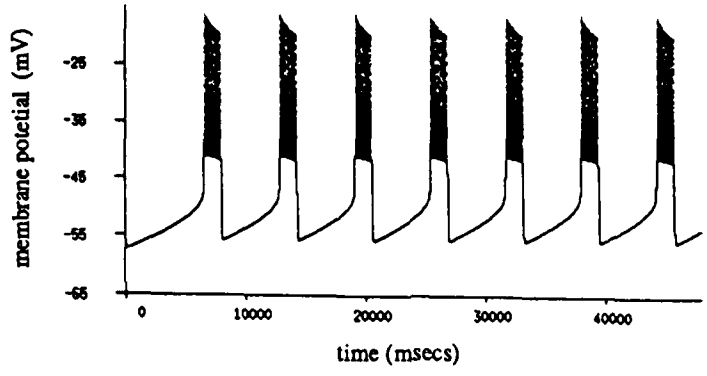

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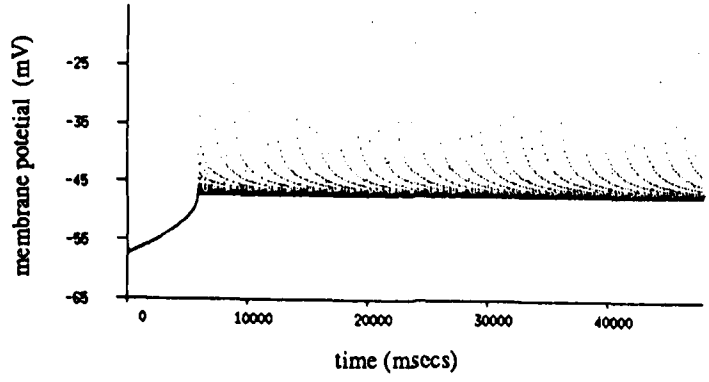

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Figure 2

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